Quantitative Lateral Flow Kit for Detection of CP4 EPSPS (RUR)® in Bulk Grain Soybean and Canola Using RapidScan ST5 Lateral Flow Reader

Cat No. EAID 101

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1. Intended Use

This lateral flow strips (LFS) test kit is intended to be used for quantitative detection of CP4 EPSPS protein in Roundup ready (RUR)® bulk grain soybean or canola samples. The sensitivity of these strips for soybean is 0.05% (i.e. detects one Roundup Ready soybean in 2000 conventional soybeans) & for canola is 0.1% (i.e. detects one Roundup Ready canola kernel in 1000 conventional canola kernel). The total incubation time of the assay is 5 minutes.

2. Principle of the Test

When the LFS is placed in the sample extract, the CP4 EPSPS protein present in the sample extracts binds to the antibody labelled with small volume of gold nanoparticles and the complex moves upward by capillary action. The complex then binds to the antibody coated on the test line resulting in pink/purple color test line. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In absence of CP4 EPSPS protein, the test line does not appear as no complex binds to the test line while control line turns pink/purple color indicating validity of test protocol. The intensity of test line can be used to quantitatively measure the amount of CP4 EPSPS present in the grain samples.

The strips may then be scanned and the results are interpreted quantitatively with the help of RapidScan ST5 Lateral Flow Reader.

Note: The standard curve for the quantitative determination of CP4 EPSPS was derived from reference material. Actual field expression may vary.

3. Cross Reactivity

The Quantitative LFS kit for detection of CP4 EPSPS kit does not cross react with Cry1F, Cry1Ac, Cry1Ab, Cry2A, Vip3A, Cry34Ab, Cry35Ab1, Cry3B, mCry3A, eCry3.1Ab and PAT (Liberty Link***)®.

4. Contents of the Kit:

4.1. Kit is Sufficient for 100 Tests

- CP4 EPSPS LFS strips : 50 strips per canister.
- Canisters per kit : 2 Nos
- Pack Insert : 1 No.
- Microfuge tubes : 100 Nos
- Transfer pipettes : 100 Nos

4.2. Material and equipment's required but not provided

- Weighing balance
- Domestic/ Bunn grinder
- Graduated cylinder (50 ml)
- Glass Jars
- Water
- Scissors
- Timer
- RapidScan ST5 Lateral Flow Assay Reader for Quantitative analysis (Eurofins Technologies, Article No.: LFRSCAN002).
- RapidScan ST5 Reader Adapter for GMO strips (Eurofins Technologies, Article No.: LFA0060001).

5. Precautions

The CP4 EPSPS LFS kit is intended for *in vitro* use only. The reagents contain Sodium azide as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

6. Storage of the Kit

The kit should be stored under refrigeration at 2 to 8 ^{o}C. The unopened kit is stable till the expiry date printed on the kit label. The cap of the canister should be closed firmly after removing the required strips. Exposure to moisture is likely to affect the performance of the test strips.

The kit should not be frozen.

7. Sample Preparation

7.1. Weigh soybeans (Approximate weight of one soybean is 0.15 gm) or canola into appropriate size jar.

For soybean samples:

No. of Beans (approximate)	Blender type	Type of grinding	Sub-sample weight (gm)	Jar size (oz.)	Grind time at high speed
100 - 200	Domestic grinder	Dry	15 - 30	16	2 X 30 Sec
200 - 400	Domestic grinder	Dry	30 - 60	32	2 X 45 Sec

For canola samples:

No. of Kernels (approximate)	Blender type	Type of grinding	Sub-sample weight (gm)	Jar size (oz.)	Grind time at high speed
800-1000	Domestic grinder	Dry	4- 5	16	2 X 30 Sec
1600-2000	Domestic grinder	Dry	8 - 10	32	2 X 45 Sec

7.2. Place cover on the jar and grind it in a blender on high speed as mentioned in the above table or till fine grain powder is observed (by using a domestic grinder or equivalent grinding method, grind a particular batch/lot sample for testing, 70-80% of the sample should be able to pass through a 20 mesh sieve).

7.3. Add required quantity of water to the jar as mentioned below.

Soybean extraction:

Grams of soybean x 7 = ml of water

For example: 150 g x 7 = 1050 ml of water

Canola extraction:

Grams of canola x 5 = ml of water

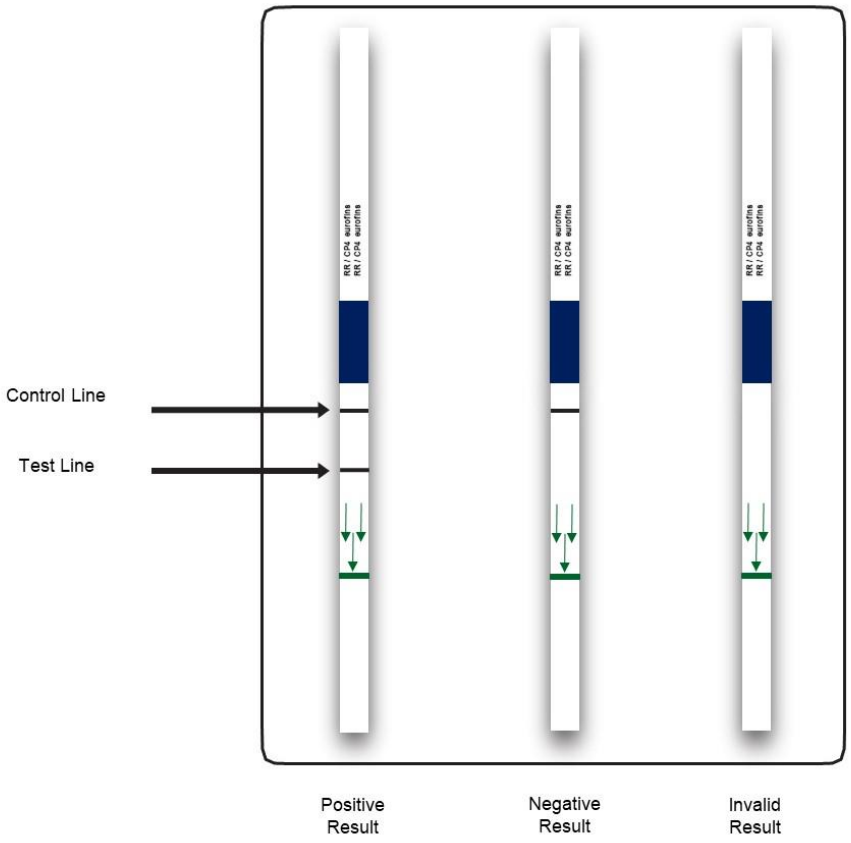
For example: 150 g x 5 = 750 ml of water

7.4. Shake jar vigorously till entire sample is properly mixed. Allow sample to settle following which liquid from top can be collected. Draw ~ 0.5 ml sample using transfer pipettes and transfer it to 1.5 ml microfuge tubes.

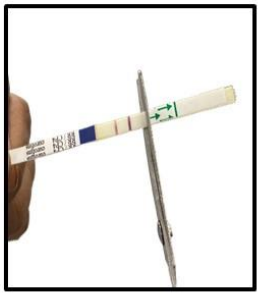
7.5. Use new dropper and microfuge tubes for each sample to avoid cross contamination.

8. Assay Procedure

8.1. Allow canister to come to room temperature before opening it to remove the desired number of strips for testing. Do not bend the strips.
8.2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the microfuge tube in vertical position for 5 minutes.
8.3. Remove the strip and observe the result. Positive sample result may appear much earlier than 5 minutes.
8.4. The appearance of one line (control line) indicates a negative test result.
8.5. Cut off the bottom section of the strip covered with arrow using scissors.
8.6. Place the top white colored strip into the RapidScan ST5 LF reader for quantitative analysis.
Note: Make sure that there is no cross-contamination with other grains or fluids.




Lateral Flow Strip In Sample


Cutting Of Strip For Permanent Record Storage and Read In RapidScan System

9. Interpretation of LFS Results

9.1. Read the strip in 5 minutes.
9.2. Presence of control line in 5 minutes indicates that the strip has performed properly. The absence of control line in 5 minutes makes test invalid and should be repeated.
9.3. If the extract is from soybean sample containing at least 0.05% RUR (one RUR soybean in 2000 conventional seeds), the test line will appear.
9.4. If the extract is from canola sample containing at least 0.1% RUR (one RUR canola kernel in 1000 conventional seeds), the test line will appear.
9.5. For quantitative analysis, the results are scanned and interpreted in RapidScan ST5 LF reader.
9.6. Place the lateral flow strip on the adapter of the reader, slide in and press “start analysis” on the screen. Quantitative results will be obtained with the RapidScan system.
9.7. RapidScan reader will show the quantitative result for the test strip as “% GMO (< LOQ (0.05 %), 0.05 % to 5 % or > 5%) for soybean samples.
9.8. RapidScan reader will show the quantitative result for the test strip as “% GMO (< LOQ (0.1 %), 0.1 % to 5 % or > 5%) for canola samples. For more details, refer to RapidScan user manual.

10. Cleaning and Maintenance of grinding equipment

10.1. It is important to clean and dry the grinding jars and cutting blades between sample batches.
10.2. The grinding jars should be emptied without leaving any powder residuals in between the usage.
10.3. The cutting blades and grinder jars should be rinsed with running water until all ground seed powder (soybean or canola) is removed.
10.4. Wash using liquid detergent and rinse well, carefully dry with paper towels if necessary.
10.5. Cross contamination between different samples during sample preparation will lead to wrong results.

11. Notes

11.1. The procedure instructions should be strictly followed to get correct results. Change in procedure may lead to wrong results.
11.2. The kit is intended for testing on soybean and canola samples only (working samples: extracted bulk soybean or canola samples).
11.3. This kit is not meant for exact percentage screening of CP4 EPSPS (RUR)® in GMO soybeans or canola, the kit protocol only gives the probability that particular batch/lot contains a certain threshold concentration above or below 0.05% for soybean and 0.1% for canola quantitatively when used with the RapidScan ST5 lateral flow reader.
11.4. Experienced laboratory technologist may be able to observe a faint test line at level below 0.05% for soybean and 0.1% for canola.
11.5. When in doubt, please confirm results with an alternate method.

11.6. Protect all the kit components from the extreme temperature when not in use.
11.7. The strip is not recommended for visual interpretation of results. The result is obtained only through RapidScan ST5 LF reader.

12. WARRANTY

Eurofins Amar Immunodiagnostics Pvt. Ltd. warrants that the products sold hereunder ("the Product") are defect free in material and workmanship, provided they are used in accordance with the prescribed instructions before the expiry of the products as printed on the product label. The customer should notify Eurofins Amar Immunodiagnostics in writing of Warranty defects during the warranty period, including an offer by the customer to return the Products to Eurofins Amar Immunodiagnostics for evaluation. Eurofins Amar Immunodiagnostics will repair or replace, at its sole option, any product or part thereof that proves defective in materials or workmanship within the warranty period. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Eurofins Amar Immunodiagnostics.

13. THIS WARRANTY IS EXCLUSIVE

The sole and exclusive obligation of Eurofins Amar Immunodiagnostics shall be to repair or replace the defective Products in the manner and for the period provided above. Eurofins Amar Immunodiagnostics shall not have any other obligation or liability, whatsoever it may be, with respect to the Products or any part thereof. Under no circumstances, whatsoever the circumstances may be, shall Eurofins Amar Immunodiagnostics be liable for incidental, special, or consequential damages. If any part of this Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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**Bollgard & Roundup Ready are registered trademarks of the Monsanto Company.

***Liberty Link is a register trademark of Bayer Crop Science.

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